Wolters Kluwer Health | Lippincott Williams & Wilkins

The American Journal of Forensic Medicine and Pathology

 Volume 18(1), March 1997, pp 45-49

Bayesian Evaluation of the Modified Zinc Test and the Acid Phosphatase Spot Test for Forensic Semen Investigation

Hooft, Peter J. M.D., M.P.H., Ph.D.; van de Voorde, Herman P. M.D., M.P.H., Ph.D.

From the Centrum voor Gerechtelijke Geneeskunde, Leuven, Belgium.
Received April 24, 1995; accepted December 20, 1995.
Address correspondence and reprint requests to Dr. Peter J. Hooft, Centrum voor Gerechtelijke Geneeskunde v.z.w., Kapucijnenvoer 10, B-3000 Leuven, Belgium.

Abstract

The modified zinc test is evaluated as a screening instrument in forensic semen identification using the classical acid phosphatase test as a "gold standard" reference. Both tests were applied to vaginal swabs taken from women (n = 456) at varying time periods after sexual intercourse and the corresponding sensitivity and specificity were calculated for both tests separately and for their parallel and serial combination strategies, with the microscopical visualization of sperm as end criterion. The results were submitted to Bayesian calculations and a model was obtained, giving the posterior probabilities of finding or not finding sperm in a microscopical preparate for any possible prior probability. For the largest part of the theoretical sperm prevalence range, negative predictions were more informative than positive ones. With the prior probability of finding spermatozoa set at P = 0.5 (Bayes' postulate of neutral prior probability), the model was applied to a set of 192 pieces of evidentiary material from alleged sexual assaults.

Many preliminary and confirmatory tests have been proposed for the investigation of semen-suspected evidentiary materials in cases of alleged sexual assault (1). None of them, however, has shown to be infallible. This is also true for the classical acid phosphatase tests, as acid phosphatases from human secretions other than semen (e.g., blood, vaginal fluid) and from nonhuman sources (e.g., some vegetables, spices, and herbs) may interfere with the test, and the enzyme is sensitive to biodegradation (2-13). Even the microscopical searching for sperm heads, for a long time used as the gold standard in forensic semen investigation, may fail for a number of reasons, among which pathological or iatrogenical

azoospermia and biodegradation due to a long delay between assault and sampling or improper storage of samples.

In 1983 Suzuki et al. [14] suggested that seminal zinc could be a valuable marker for semen traces in the forensic context and a good alternative for other, more degradable markers such as acid phosphatase. A spot test was proposed based on the formation of an insoluble complex of zinc with 1-(2-pyridylazo)-2-naphthol (PAN) (14,15). In an attempt to obtain a workable, simple technique for the preliminary screening of semen-suspected stains, we modified the initial reagent and obtained a test variant that showed to be more sensitive and suitable for the development of stable test paperstrips (5,16).

This article addresses part of the evaluation of this new test, focusing on the application of epidemiological principles in the evaluation of screening tests. Other aspects of the evaluation have been addressed elsewhere (5,6).

MATERIALS AND METHODS

The screening tests used were of a qualitative nature and consisted of a commercially available acid phosphatase test paperstrip [Phosphatesmo KM (Macherey-Nagel, Düren, Germany)] and the zinc test paperstrip we developed (16). The microscopical investigation of spermatozoa was performed on preparates stained with alcalic fuchsin after a differential cell lysis of the suspected extract with proteinase K according to Chapman (17).

In the initial study, vaginal swabs were obtained from 261 sexually active women at the occasion of a gynecological examination or pregnancy checkup. During the anamnesis, the physician asked for the approximate time period since last vaginal sexual intercourse. Women reporting the use of spermicidal products or a condom were excluded, as were those who reported menstrual bleeding in the time period between last intercourse and sampling. Later on, the study was repeated on vaginal swabs taken from 195 other women, for whom it was additionally known that they lived a regular monogamous life with a fertile partner. All swabs were transferred to the laboratory in a dry state and the investigators were not informed of the anamnestic data until the swabs had been processed.

On investigation, the swabs were moistened with two drops of physiological saline and the screening test paperstrips were pressed on the moistened swab in a randomly changed order. The color reaction was noted after 1 minute. The swab was then extracted in physiological saline and a microscopical preparate was made after treatment of the extract with proteinase K.

The data for the evaluation of the predictive model derived from the vaginal swabs studies were obtained from the investigation of 192 pieces of evidentiary material in cases of alleged sexual assault. These mainly consisted of vaginal swabs or washings (49%), underwear (30%), clothing (12%), and bed sheets (4%). For analysis purposes, all samples were regarded as being independent even if they came from the same case. The screening test paperstrips were applied to the moistened objects in a random order and the color reaction was noted after 1 minute. From each piece of evidentiary material a microscopical preparate was made, starting from a proteinase K-treated extract.

In the analysis, the classical qualitative acid phosphatase test was used as the "gold standard" reference and the visualization of spermatozoa as the end criterion. As earlier findings suggested that a screening battery based on multiple tests might well be the

method of choice (18) and a combined screening test kit for zinc and acid phosphatase has recently become commercially available (12), the analysis was expanded to include the parallel and serial combinations of both screening tests. In a parallel combination strategy, the final result is said to be positive if at least one of the tests is positive, whereas in a serial combination strategy all tests have to be positive before the final result is said to be positive (19). Traditionally, parallel combination of test results raises the sensitivity at the inevitable cost of lowering specificity, whereas serial combination has an opposite effect.

RESULTS

Figure 1 shows the sperm prevalence in microscopical preparates according to the time period since last sexual intercourse in both vaginal swabs studies. The overall sperm prevalence was 36% in the first study and 35% in the second. This small and statistically not significant difference may have arisen from different numbers of women with recent sexual intercourse: in the initial group, 21% reported intercourse within the last 24 h and 14% within 24-48 h before sampling, against 16% and 13% in the second group. The percentage of positive microscopical findings in samples taken after recent intercourse, on the other hand, was higher in the second group: 87% versus 80% for the 24 h sampling window and 68% versus 63% for the 24-48 h sampling window (difference statistically not significant). This finding may possibly be explained by the correction for eventual azoic partners present in the initial group, as this factor was corrected for only in the second study. In samples taken after longer abstinence periods (5 days or more), the rate of sperm positivity was lower in the second group of women. The hypothesis that some women from the first study, where no selection had been made as to the sexual lifestyle had, for one or another reason, falsely reported a long abstinence period, was occasionally confirmed by a microscopical picture of rather "fresh" sperm heads. However, it was felt that this bias was of minor importance to the object of the present study and the data from both groups were taken together for further calculations.

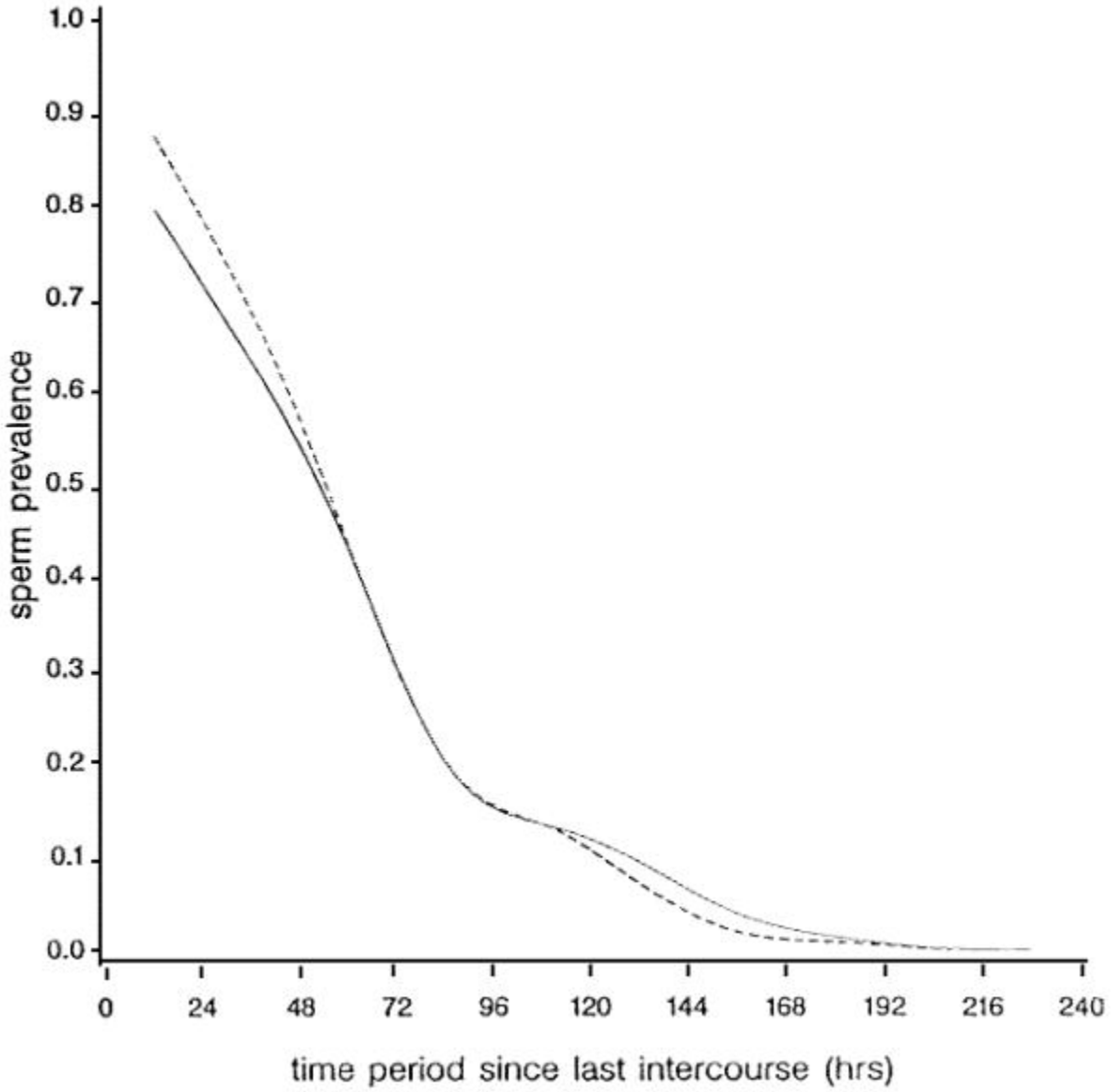

FIG. 1. Sperm prevalence in microscopical smears made from vaginal swabs after different time periods since last sexual intercourse. Solid line: sexually active women (n = 261). Dashed line: sexually active women living a regular monogamous life with a fertile partner (n = 195).

For both vaginal swabs studies taken together, Table 1 gives the overall sensitivity and specificity of the zinc test and the acid phosphatase test separately and of their parallel and serial combinations. Starting from these parameters, Figs. 2 and 3 for all possible prior probabilities of finding spermatozoa in microscopical preparates show the evolution in the positive predictive values (i.e., the probability of finding spermatozoa given a positive screening test result, or positive posterior probability) and the negative predictive values (i.e., the probability of not finding spermatozoa given a negative screening test result, or negative posterior probability). When the prior probability of visualizing spermatozoa is set at the highest uncertainty level [equal chances on finding or not finding spermatozoa (P =

0.5)], the positive predictive values are 0.87, 0.80, 0.78, and 0.90, respectively, for the zinc test, the acid phosphatase test, the parallel combination strategy, and the serial combination strategy. The negative predictive values under that condition are 0.98, 0.89, 0.99, and 0.90, respectively.

Test	Sensitivity (CL95)	Specificity (CL95)
Zinc	0.98 (0.96 – 1.00)	0.86 (0.82 – 0.90)
Acid phosphatase	0.91 (0.87 – 0.95)	0.77 (0.72 – 0.82)
Parallel combination	0.99 (0.98 – 1.00)	0.72 (0.67 – 0.77)
Serial combination	0.90 (0.85 – 0.95)	0.90 (0.87 – 0.93)

TABLE 1. Sensitivity and specificity (and 95% confidence interval) of tests of vaginal swabs taken after different time periods since last sexual intercourse (n = 456).

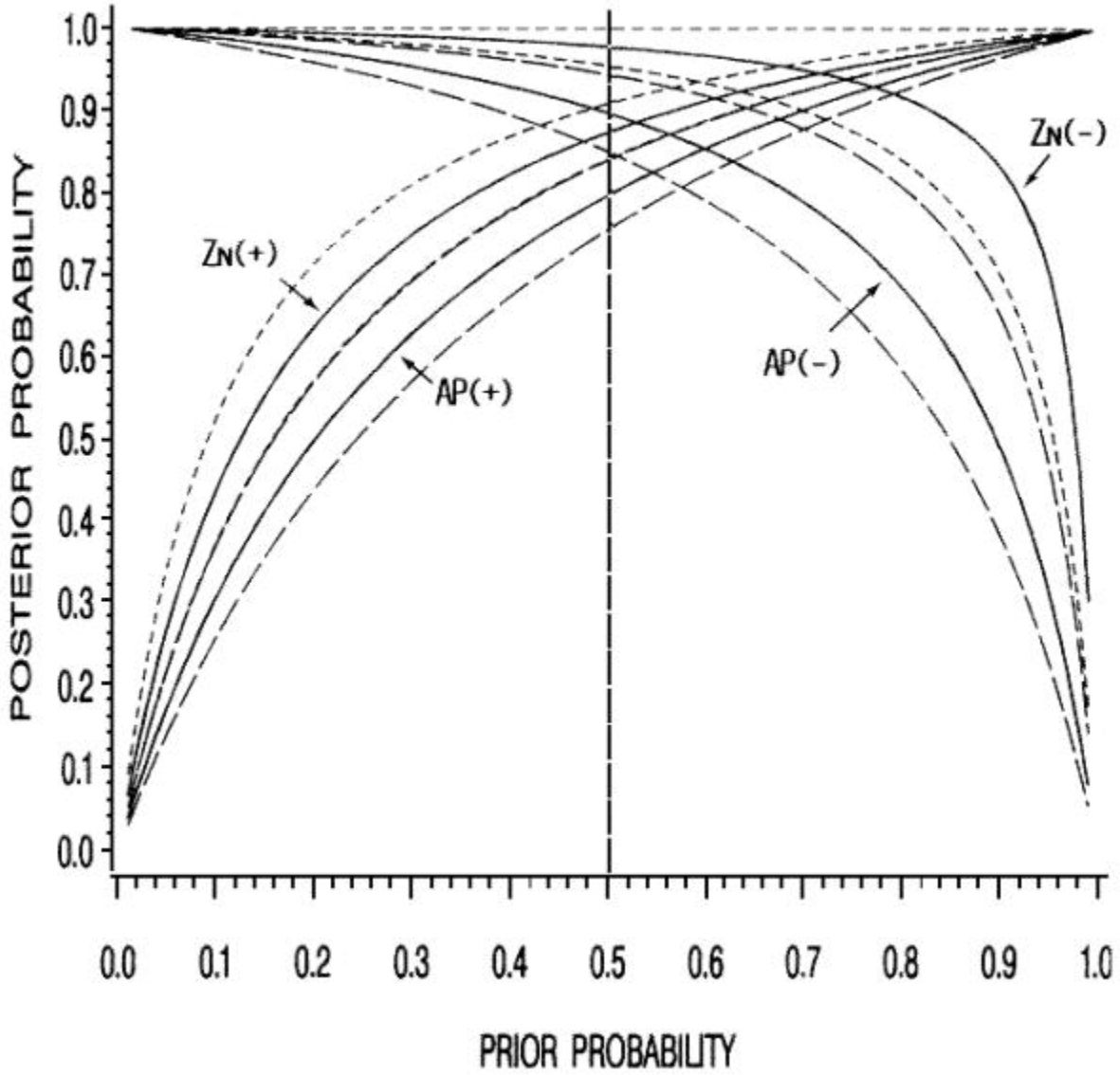

FIG. 2. Probability of (not) finding spermatozoa in microscopical preparates according to the foregoing screening test result and the prior probability of microscopical visualization of spermatozoa. Solid lines: predictions from the point-estimated sensitivities and specificities; dashed lines: predictions from the lower and the higher CL95 values of the sensitivities and specificities. ZN(+), probability of finding spermatozoa given a positive zinc test; AP(+), probability of finding spermatozoa given a positive acid phosphatase test; ZN(-), probability of not finding spermatozoa given a negative zinc test; AP(-), probability of not finding spermatozoa given a negative acid phosphatase test. Reference line: equal prior probability of finding and not finding spermatozoa.

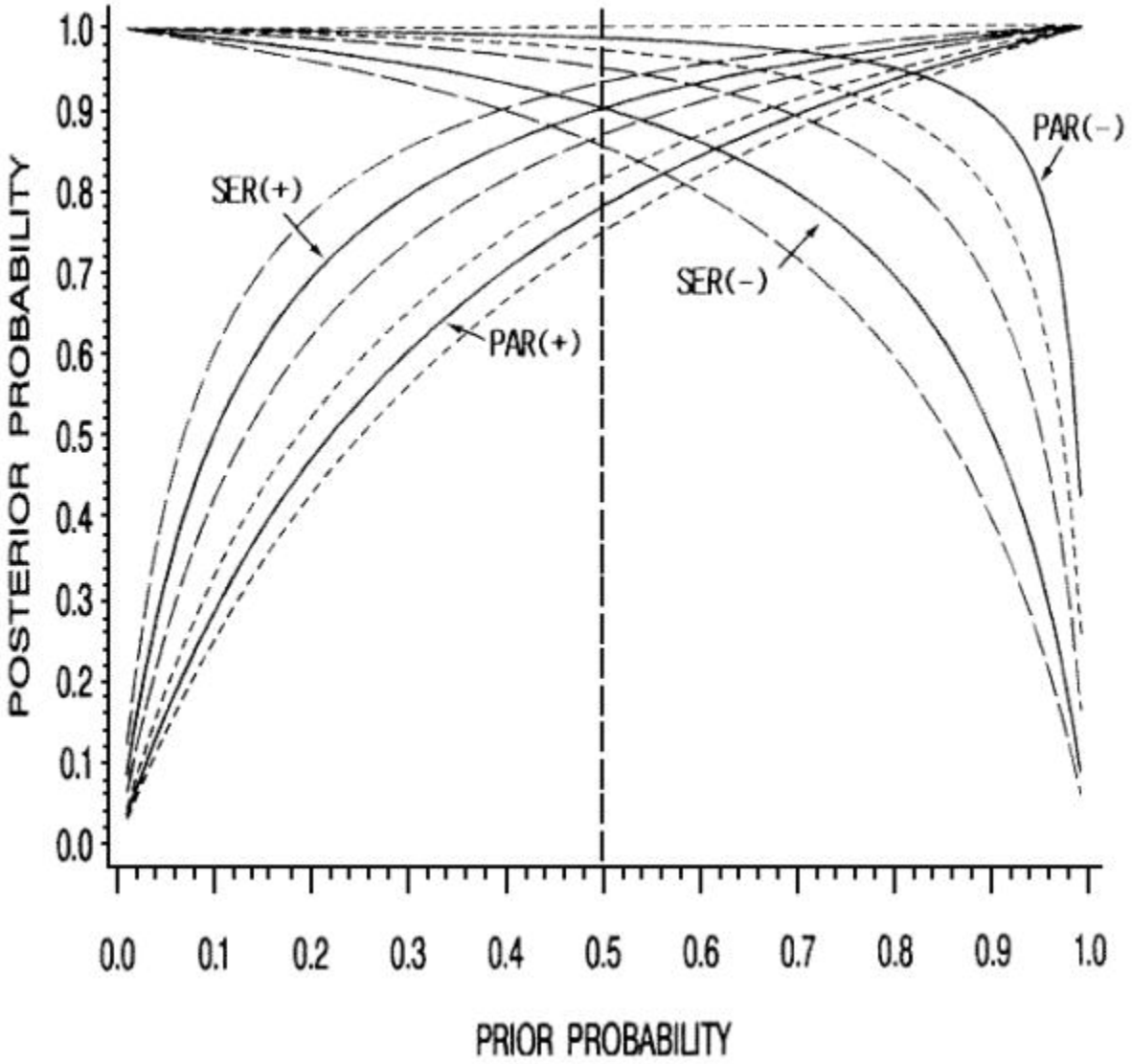

FIG. 3. Probability of (not) finding spermatozoa in microscopical preparates according to the foregoing screening test result and the prior probability of microscopical visualization of spermatozoa. Solid lines: predictions from the point-estimated sensitivities and specificities; dashed lines: predictions from the lower and the higher CL95 values of the sensitivities and specificities. PAR(+), probability of finding spermatozoa given a positive zinc or acid phosphatase test; SER(+), probability of finding spermatozoa given a positive zinc and acid phosphatase test; PAR(-), probability of not finding spermatozoa given a negative zinc and acid phosphatase test; SER(-), probability of not finding spermatozoa given a negative zinc or acid phosphatase test. Reference line: equal prior probability of finding and not finding spermatozoa.

Table 2 summarizes the findings in the pieces of evidentiary material from alleged sexual assaults. From the model, fairly good predictions were obtained of what could be expected at microscopical investigation, but the positive predictive values showed to underestimate the number of microscopically positive samples irrespective of the test strategy. The negative predictive values led to very good predictions of the number of microscopically

negative samples and this prediction was correct even for the parallel and serial test combination strategies.

Screening result	N	Microscopical results: Predicted	Microscopical results: Observed
		Positive	
Zinc +	113	98	101
Acid phosphatase +	119	95	97
Parallel combination +	128	100	103
Serial combination +	104	94	95
		Negative	
Zinc −	79	77	76
Acid phosphatase −	73	65	66
Parallel combination −	64	63	63
Serial combination −	88	79	79

*The prediction is obtained by multiplying N with the positive or the negative posterior probability, respectively, obtained at a prior probability of 0.5 from Figs. 2 and 3.

TABLE 2. Screening test results and predicted or observed microscopic findings in evidentiary material from alleged sexual assault cases (n = 192)*

DISCUSSION

Although sensitivity and specificity are both important parameters in the evaluation of screening tests, their evidentiary meaning in forensic practice often is not. This is especially true for semen detection tests when microscopy is taken as the end criterion. First, the microscopical finding of spermatozoa inevitably implies the presence of semen and, although no forensic investigator would look for the confirmation of a positive microscopical result by performing some other test, the sensitivity reflects the probability of a positive test in samples with a positive microscopical result [Se = P(T+ given M+)]. Second, zinc and acid phosphatase are constituents of the seminal plasma and spermatozoa may not be present in a semen sample owing to a number of reasons. The specificity of the tests under study, therefore, is not exclusively related to the bias originating from nonseminal zinc and acid phosphatases or other interfering elements but also to the black number of negative microscopical findings in semen-bearing specimens, making the contents of the specificity [Sp = P(T- given M-)] ambiguous. As semen detection tests should only be regarded as first filters to distinguish the samples holding high expectations from those that probably will be negative, the evaluation should focus on the ability to rightfully predict the outcome of the end criterion [P(M+ given T+) and P(M- given T-)]. For this, however, the prevalence of the end criterion should be known or at least fairly well estimated.

In the present study, two approaches have been used to deal with the latter problem. The first was to calculate the predictive values of the tests under study for all possible prior probabilities of finding spermatozoa. The zinc test then shows to be superior to the acid phosphatase test, as both its positive and negative predictive values are systematically higher. In addition, up to a prior probability of finding spermatozoa of ~72%, a negative zinc test result is more predictive than a positive one. When test combinations are taken into consideration, the negative predictive values of the parallel combination of the zinc and acid phosphatase tests are higher than those of the zinc test alone. One could therefore conclude that for the greatest part of the theoretical prior probability range of finding spermatozoa, a parallel combination of the tests that focuses on negative results (exclusion strategy) may be the method of choice. Only when the prior probability of finding spermatozoa is felt to be very high (e.g., because of another preliminary selection of samples by other methods), a serial test combination focusing on positive results (inclusion strategy) may be an alternative, although the gain is minimal when compared with the zinc test alone. The second approach has been to set the prior probability of finding spermatozoa at the highest uncertainty level of P = 0.5 (Bayes' postulate of neutral prior probability). This situation, with equal chances of finding or not finding spermatozoa, falls within the range where the parallel combination of the tests focusing on negative results (exclusion strategy) is the most predictive. The application of this model to a set of evidentiary materials from alleged sexual assaults showed that the predictions were fairly good and that the negative predictions from the test combination strategies were correct.

Acknowledgments: The authors wish to thank Dr. G. Donders, Dr. J. van Bettinghe, and Dr. H. Kaerels, gynecologists, for their collaboration during the vaginal swabs studies.

REFERENCES

1. Gaensslen R. Sourcebook in forensic serology, immunology and biochemistry. Washington, DC: US Department of Justice: National Institute of Justice, 1983;155-78.

2. Davies A, Wilson E. The persistence of seminal constituents in the human vagina. Forensic Sci Int 1974;3:45-55.

3. Hauck G, Leithoff H. Die Phosphatasebestimmung als gerichts-medizinischer Spermanachweis. D Z Gesamte Gerichtl Med 1959;49:5-20.

4. Hooft P, van de Voorde H. Comparative study of the sensitivity and specificity of the zinc and acid phosphatase spot tests for the detection of seminal stains. Z Rechtsmed 1990;103:581-6.

5. Hooft P. Seminal zinc as a screening instrument for the preliminary identification of semen traces. Acta Biomedica Lovaniensia No. 55. Leuven: Leuven University Press, 1992;1-133.

6. Hooft P, van de Voorde H. Interference of body products, food and products from daily life with the modified zinc test and the acid phosphatase test. Forensic Sci Int 1994;66:187-96.

7. Kaye S. The acid phosphatase test for seminal stains, a study on the reliability of aged stains. J Crim Law Criminol 1951;41:834-5.

8. Kempe C. Possible interference in the acid phosphatase tests for seminal fluid stains. J Crim Law Criminol Police Sci 1958;49:393-4.

9. Kind S. The acid phosphatase test. In: Curry A, ed. Methods of forensic science, vol III. New York: Interscience, 1964;267-88.

10. Sensabaugh G. The quantitative acid phosphatase test, a statistical analysis of endogenous and postcoital acid phosphatase levels in the vagina. J Forensic Sci 1979;24:346-65.

11. Shiff A. Reliability of the acid phosphatase test for the identification of seminal fluid. J Forensic Sci 1978;23:833-44.

12. Steinman G. Rapid spot tests for identifying suspected semen specimens. Forensic Sci Int 1995;72:191-7.

13. Walther G, Hohn P. Aktivitätsbestimmung der sauren Phosphatase in verschiedenen biologischen Medien unter Verwendung von o-Carboxyphenylphophat. Z Rechtsmed 1971;69:259-62.

14. Suzuki O, Asano M, Kido A, Oya M. Zinc test as a new tool for identification of human seminal stains. Forensic Sci Int 1983;22:231-5.

15. Carter P. Spectrophotometric submicrogram zinc assay: an application for the routine service laboratory. Clin Chim Acta 1974;52:277-86.

16. Hooft P, van de Voorde H, van Dijck P. A more sensitive modification of the zinc test for seminal traces suitable for stable test paper strips. Forensic Sci Int 1992;53:131-3.

17. Chapman R, Brown N, Keating S. The isolation of spermatozoa from sexual assault swabs using Proteinase K. J Forensic Sci Soc 1989;29:207-12.

18. Hooft P, van de Voorde H. Evaluation of the modified zinc test and the acid phosphatase test as preliminary screening methods in sexual assault case material. Forensic Sci Int 1992;53:135-41.

19. Bouter L, van Dongen M. Epidemiologisch onderzoek: opzet en interpretatie. Utrecht: Bohn, Scheltema & Holkema, 1988;270-4.

Key Words: Acid phosphatase; Bayes; Biological stains; Forensic medicine; Screening; Semen; Zinc